IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of : ALLEN et al.

Serial No. 09/884,514 **Group Art Unit 1648**

Filed : June 19, 2001

Examiner : S. Foley

DECLARATION PURSUANT TO 37 C.F.R SECTION 1.132

Hon. Commissioner of Patents
and Trademarks
Washington D.C. 20231

Sir :

I, Catherine Charreyre, solemnly declare :

1 - that I am one of the inventors of the above-cited application

2 - that vaccination experimentation was done under my supervision using inactivated PCV-2 having a titer before inactivation of $10^{6.6}$ TCID50 formulated in an oil-in-water emulsion. The volume for one dose of vaccine is 2 ml.

16 piglets (14 days old) have been allocated in two groups of 8 animals each, one as a control group the other as a vaccinated group. The 8 animals of the vaccinated group were injected by intramuscular route at days 0 and 21. For control group piglets were injected with a physiological solution.

Vaccinated and control animals were then challenged day 35 by oronasal administration of a PCV-2 viral suspension, 5 ml in each nostril (5.5 DICC50 per nostril).

Antibodies:

Antibodies in sera was measured by immunofluorescence.

		Day -8	Day -2	Day 19	Day 28	Day 33	Day 43
vaccinated	mean titre	2.5	2.2	1.9	2.7	2.8	2.9
	std	0.17	0.25	0.0	0.37	0.36	0.28
control	mean titre	2.4	2.3	1.9	1.9	1.9	1.9
	std	0.11	0.20	0.0	0.0	0.0	0.0

std = standard deviation
residual titre of maternal antibodies at days –8 and –2.

Vaccinated piglets seroconverted after second vaccine injection. The difference between vaccinated and control groups is significant (ANOVA analysis).

Viral excretion in feces:

Rectal swabs were collected at different time after challenge to follow viral excretion. The faecal swabs are assessed by PCR for the presence of PCV-2. Unvaccinated controls are negative for PCV-2 prior challenge and positive after challenge confirming the validity of the PCR assay.

Value are expressed as percentage of piglets excreting PCV-2 in feces and as mean duration of excretion expressed in days.

	control	vaccinated
% viral excretion	100 %	38 %
Mean duration of excretion	9.1 days	1.3 day

A reduction of viral excretion is observed in the vaccinated group. The difference between vaccinated and control piglets is significant (ANOVA analysis).

Virus load in lymph nodes tissues:

Mediastinal and mesenteric lymph nodes were collected. Virus load was determined by immunochemistry.

The data presented correspond

- **(1)** to the percentage of piglets having mediastinal or mesenteric lymph nodes from which it was possible to detect the presence of PCV-2, and

- **(2)** to the mean of scores using the following criteria :

  0 = lack of fluorescence
  1 = some fluorescent foci on some organ slides
  2 = approximately 1 foci per shot
  3 = wholly fluorescent organ.

		vaccinated	control
(1)	Mediastinal	75 %	100 %
	Mesenteric	25 %	100 %
(2)	Mediastinal	0.4	1.9
	Mesenteric	0.1	1.9

Values of **(1)** and **(2)** are lower in vaccinated piglets than in the controls. The differences are significant (Chi2 test for **(1)** and Kruskall-Wallis test for **(2)**).

Necropsy lesions:

Days 63 and 64 necropsies were performed and the lesions were scored according to the criteria appearing on appendix 1 (the score for one piglet is equal to the sum of the scores corresponding to each organ observed).

Control	16.8
vaccinated	8.9

A significant reduction (Kruskall-Wallis test) is observed in the vaccinated group.

Conclusion :

Vaccination with inactivated PCV-2 protected pigs against challenge as substantiated by significant reduction of viral excretion in feces, of virus load in organs and of lesions of PMWS.

3 – that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true ; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and of any patent issuing thereon.

Date : 06 July 2001

Name : Catherine Charreyre

Signature :

Appendix 1

Scores for macroscopic lesions

skin (color)	normal	0
	white	1
	yellow	2
corpulence	normal	0
	thin	1
	very thin	2
	cachectic	3
mucous	normal	0
	white	1
	yellow	2
sub.cut. Conjonctif	normal	0
	brillant	1
	yellow	2
ganglions (gg)	normal	0
	1 large and or congestive	1
	> 1 large and or congestive	2
	> 1 very large	3
thoracic fluide	normal	0
	brillant	1
	visible	2
heart	normal	0
	lesion	1
lungs	normal	0
	lesion ≤ 4	1
	lesion > 4 ≤ 6	2
	lesion > 6	3
pleura	normal	0
	lesion	1
ascite	normal	0
	brillant	1
	visible	2
peritoneum	normal	0
	lesion	1
stomach	normal	0
	lesion	1
	ulcer	2
small intestine	normal	0
	lesion	1
large intestine	normal	0
	lesion	1
Peyers plaques	normal	0
	visible on 1 part of the intestine	1
	visible on 2 part of the intestine	2
	very importante	3
liver	normal	0
	lesion	1
kidney	normal	0
	lesion	1
bladder	normal	0
	lesion	1